様式第１９

学 会 等 発 表 実 績

委託業務題目「造血細胞移植における免疫応答解析」
機関名　　東京女子医科大学

１．学会等における口頭・ポスター発表

発表した成果 （発表題目、口頭 ・ポスター発表の	発表者氏名	発表した場所 （学会等名）	発表した時期	国内・ 外の別
造血細胞移植の移植免疫学 ーNK細胞の役割ー	田中淳司	第76回日本血液学会教育講演	2014. 10. 31	国内

２．学会誌・雑誌等における論文掲載

掲載した論文（発表題目）	発表者氏名	発表した場所 （学会誌・雑誌等名）	発表した時期	国内・ 外の別
Etoposide-containing conditioning regimen reduces the occurrence of hemophagocytic lymphohistiocytosis after SCT.	Kobayashi R, Tanaka J, Hashino S, Ota S, Torimoto Y, Kakinoki Y, Yamamoto S, Kurosawa M, Hatakeyama N, Haseyama Y, Sakai H, Sato K, Fukuhara T.	Bone Marrow Transplant.	2014	国外
Epstein-barr virus-associated smooth muscle tumors after bone marrow transplantation.	Hayase E, Fujimoto K, Mitsuhashi T, Hatanaka Y, Yoshida M, Takemura R, Iwasaki J, Shiratori S, Sugita J, Kondo T, Tanaka J, Imamura M, Matsuno Y, Teshima T.	Transplantation.	2014	国外
Bone Marrow Graft-versus-Host Disease: Evaluation of Its Clinical Impact on Disrupted Hematopoiesis after Allogeneic Hematopoietic Stem Cell Transplantation.	Shono Y, Shiratori S, Kosugi-Kanaya M, Ueha S, Sugita J, Shigematsu A, Kondo T, Hashimoto D, Fujimoto K, Endo T, Nishio M, Hashino S, Matsuno Y, Matsushima K, Tanaka J, Imamura M, Teshima T.	Biol Blood Marrow Transplant.	2014	国外
Pre-transplant administration of imatinib for allogeneic hematopoietic stem cell transplantation in patients with BCR-ABL-positive acute lymphoblastic leukemia.	Mizuta S, Matsuo K, Imai K, Nishiwaki S, kanamori H, Ohashi K, Fukuda T, Onishi Y, Miyamura K, Takahashi S, Onizuka M, Suzuki R, Atsuta Y, Morishima Y, Kato K, Sakamaki H, Tanaka J.	Blood	2014	国外
Donor lymphocyte infusion for the treatment of relapsed acute myeloid leukemia after allogeneic hematopoietic stem cell transplantation : A retrospective analysis by the Adult AML Working Group of the Japan Society for Hematopoietic Cell Transplantation.	Takami A, Yano S, Yokoyama H, Kuwatsuka Y, Yamaguchi T, Kanda Y, Morishima Y, Fukuda T, Miyazaki Y, Nakamae H, Tanaka J, Atsuta Y, Kanamori H.	Biol Blood Marrow Transplant.	2014	国外

様式第１９

学 会 等 発 表 実 績

委託業務題目「ハプロ一致移植における骨髄由来培養間葉系幹細胞の有用性の検討」
機関名　　名古屋大学

１．学会等における口頭・ポスター発表

発表した成果（発表題目、口頭 ・ポスター発表の別）	発表者氏名	発表した場所（学会等名）	発表した時期	国内・外の別
Hematopoietic stem cell transplantation from an alternative donor for childhood aplastic anemia: HLA haploidentical family donor vs HLA mismatched unrelated donor	Takahashi Y.	40th Annual Meeting of the EBMT.	2014/4/9	国外
造血細胞移植後にみられる感染症に対する治療の進歩	高橋 義行	第117回日本小児科学会学術集会	2014/4/11	国内
Unmanipulated HLA haploidentical bone marrow transplantation combined with PBSC using high dose ATG	高橋 義行、関屋 由子、川島 希、成田 敦、土居崎 小夜子、奥野 友介、入江 正寛、村松 秀城、濱 麻人、小島 勢二.	第76回日本血液学会学術集会	2014/10/31	国内
KIR ligand incompatible allogeneic cord blood transplantation for high risk neuroblastoma as an KIR mismatched NK cell immunotherapy. 難治性神経芽腫に対するＫＩＲリガンド不一致性同種臍帯血移植を用いたアロＮＫ細胞免疫療法の試み	高橋 義行	第56回日本小児血液・がん学会学術集会	2014/11/30	国内

２．学会誌・雑誌等における論文掲載

掲載した論文（発表題目）	発表者氏名	発表した場所（学会誌・雑誌等名）	発表した時期	国内・外の別
Haematopoietic stem cell transplantation for relapsed or refractory anaplastic large cell lymphoma: a study of children and adolescents in Japan.	Fukano R, Mori T, Kobayashi R, Mitsui T, Fujita N, Iwasaki F, Suzumiya J, Chin M, Goto H, Takahashi Y, Hara J, Park YD, Inoue M, Koga Y, Inagaki J, Sakamaki H, Adachi S, Kawa K, Kato K, Suzuki R.	Br J Haematol	2015	国外
Choreito formula for BK virus-associated hemorrhagic cystitis after allogeneic hematopoietic stem cell transplantation.	Kawashima N, Ito Y, Sekiya Y, Narita A, Okuno Y, Muramatsu H, Irie M, Hama A, Takahashi Y, Kojima S.	Biol Blood Marrow Transplant	2015	国外

Comparison of continuous and twice-daily infusions of cyclosporine A for graft-versus-host-disease prophylaxis in pediatric hematopoietic stem cell transplantation.	Umeda K, Adachi S, Tanaka S, Ogawa A, Hatakeyama N, Kudo K, Sakata N, Igarashi S, Ohshima K, Hyakuna N, Chin M, Goto H, Takahashi Y, Azuma E, Koh K, Sawada A, Kato K, Inoue M, Atsuta Y, Takami A, Murata M.	Pediatr Blood Cancer	2015	国外
Bloodstream infection after stem cell transplantation in children with idiopathic aplastic anemia.	Kobayashi R, Yabe H, Kikuchi A, Kudo K, Yoshida N, Watanabe K, Muramatsu H, Takahashi Y, Inoue M, Koh K, Inagaki J, Okamoto Y, Sakamaki H, Kawa K, Kato K, Suzuki R, Kojima S.	Biol Blood Marrow Transplant	2014	国外
First-line treatment for severe aplastic anemia in children: bone marrow transplantation from a matched family donor versus immunosuppressive therapy.	Yoshida N, Kobayashi R, Yabe H, Kosaka Y, Yagasaki H, Watanabe K, Kudo K, Morimoto A, Ohga S, Muramatsu H, Takahashi Y, Kato K, Suzuki R, Ohara A, Kojima S.	Haematologica	2014	国外

様式第１９

学 会 等 発 表 実 績

委託業務題目「マウスモデルを使ったHLA不適合移植後の免疫寛容の誘導に関する検討」
機関名　岡山大学病院　血液・腫瘍内科

１．学会等における口頭・ポスター発表

発表した成果（発表題目、口頭 ・ポスター発表の別）	発表者氏名	発表した場所（学会等名）	発表した時期	国内・外の別
Host Tissue PD-1 Pathway Contribute To Murine Chronic Graft-Versus-Host Disease Via Th1+Th17+ Cells.	Takanori Yoshioka, Yusuke Meguri, Takeru Asano, Taro Masunari, Kumiko Kagawa, Koichi Nakase, Yoshinobu Maeda, Mitsune Tanimoto, Ken-ichi Matsuoka	American Society of Hematology 56th Annual Meeting, San Francisco	2014/12/5	国外
Use of Recombinant Thrombomodulin for Thrombotic Microangiopathy after Hematopoietic Stem Cell Transplantation Ameliorate Disease Severity	Hideaki Fujiwara, Yoshinobu Maeda, Yasuhisa Sando, Makoto Nakamura, Katsuma Tani, Takanori Ishikawa, Hisakazu Nishimori, Ken-Ichi Matsuoka, Nobuharu Fujii, Eisei Kondo, Mitsune Tanimoto	American Society of Hematology 56th Annual Meeting, San Francisco	2014/12/5	国外
Anti-IL-12/23 p40 Antibody Attenuates Chronic Graft Versus Host Disease Via Suppression of IFN-γ/IL-17-Producing Cells	Taiga Kuroi, Sachiyo Okamoto, Kyosuke Saeki, Yujin Kobayashi, Hisakazu Nishimori, Hideaki Fujiwara, Ken-ichi Matsuoka, Nobuharu Fujii, Eisei Kondo, Mitsune Tanimoto, Yoshinobu Maeda	American Society of Hematology 56th Annual Meeting, San Francisco	2014/12/5	国外
Host Immune Status Determines the Effects of Therapeutic Interleukin-2 Administration: Enhancement of GVL or Induction of Tolerance?	Yusuke Meguri, Takeru Asano, Takanori Yoshioka, Haruka Izumi, Yuriko Kishi, Yoshinobu Maeda, Mitsune Tanimoto, Ken-ichi Matsuoka	American Society of Hematology 56th Annual Meeting, San Francisco	2014/12/5	国外
Recombinant Thrombomodulin for the Treatment of Transplantation-Associated Coagulopathy after Allogeneic Hematopoietic Stem Cell Transplantation	Kazuyoshi Ishii, Shosaku Nomura, Tomoki Ito, Yuta Katayama, Taiichi Kyo, Shuichi Ota, Masanori Seki, Shigeru Chiba, Yoshinobu Maeda, Mitsune Tanimoto, Takayuki Ikezoe, Hideo Yagi, Yoji Ishida, Naohito Fujishima, Kenichi Sawada	American Society of Hematology 56th Annual Meeting, San Francisco	2014/12/5	国外

2．学会誌・雑誌等における論文掲載

掲載した論文（発表題目）	発表者氏名	発表した場所（学会誌・雑誌等名）	発表した時期	国内・外の別
Anti-IL-12/23 p40 antibody attenuates experimental chronic graft versus host disease via suppression of IFN-γ/IL-17-producing cells.	Okamoto S, Fujiwara H, Nishimori H, Matsuoka K, Fujii N, Kondo E, Tanaka T, Yoshimura A, Tanimoto M, Maeda Y.	J Immunol	in press	国外
The impact of HLA mismatch direction on the outcome of unrelated bone marrow transplantation: A retrospective analysis from the JSHCT	Kanda J, Ichinohe T, Fuji S, Maeda Y, Ohashi K, Fukuda T, Miyamura K, Iwato K, Eto T, Nakamae H, Kobayashi N, Mori T, Mori SI, Morishima Y, Atsuta Y, Kanda Y.	Biol Blood Marrow Transplant	in press	国外
Prognostic significance of pleural or pericardial effusion, and the implication of optimal treatment in primary mediastinal large B-cell lymphoma: a multicenter retrospective study in Japan.	Aoki T, Izutsu K, Suzuki R, Nakaseko C, Arima H, Shimada K, Tomita A, Sasaki M, Takizawa J, Mitani K, Igarashi T, Maeda Y, Fukuhara N, Ishida F, Niitsu N, Ohmachi K, Takasaki H, Nakamura N, Kinoshita T, Nakamura S, Ogura M.	Haematologica	in press	国外
Programmed Death-1 Pathway in Host Tissues Ameliorates Th17/Th1-Mediated Experimental Chronic Graft-versus-Host Disease.	Fujiwara H, Maeda Y, Kobayashi K, Nishimori H, Matsuoka K, Fujii N, Kondo E, Tanaka T, Chen L, Azuma M, Yagita H, Tanimoto M.	J Immunol	2014	国外
Changes in the clinical impact of high-risk HLA allele mismatch combinations on the outcome of unrelated bone marrow transplantation	Kanda Y, Kanda J, Atsuta Y, Fuji S, Maeda Y, Ichinohe T, Takanashi M, Ohashi K, Fukuda T, Miyamura K, Mori T, Sao H, Kobayashi N, Iwato K, Sawada A, Mori S.	Biol Blood Marrow Transplant	2014	国外
mTOR inhibitors permit regulatory T cell reconstitution and inhibit chronic GVHD	Sugiyama H, Maeda Y, Nishimori H, Yamasuji Y, Matsuoka K, Fujii N, Kondo E, Shinagawa K, Tanaka T, Takeuchi K, Teshima T, and Tanimoto M	Biol Blood Marrow Transplant	2014	国外

様式第１９

学 会 等 発 表 実 績

委託業務題目「生物統計的検討」
機関名　　京都大学

１．学会等における口頭・ポスター発表

発表した成果（発表題目、口頭 ・ポスター発表の別）	発表者氏名	発表した場所（学会等名）	発表した時期	国内・外の別
特記すべき発表はなし				

２．学会誌・雑誌等における論文掲載

掲載した論文（発表題目）	発表者氏名	発表した場所（学会誌・雑誌等	発表した時期	国内・外の別
Incorporating historical data in Bayesian phase I trial design: The Caucasian-to-Asian toxicity tolerability problem.	Takeda K, Morita S.	Therapeutic Innovation & Regulatory Science	in press	国外
Biomarker-based Bayesian randomized phase II clinical trial design to identify a sensitive patient subpopulation.	Morita S. Yamamoto H, Sugitani	Stat Med	2014	国外
Exploring ethnic differences in toxicity in early phase clinical trials for oncology drugs.	Ogura T, Morita S, Yonemori K, Nonaka T, Urano T.	Therapeutic Innovation & Regulatory Science	2014	国外
A continual reassessment method with cohort size adaptation based on Bayesian posterior probabilities in phase I dose-finding studies.	Kakizume T, Morita S.	Therapeutic Innovation & Regulatory Science	2014	国外
Progression-Free Survival as a Surrogate for Overall Survival in Advanced/Recurrent Gastric Cancer Trials: A Meta-Analysis.	Paoletti X, Oba K, Bang YJ, Bleiberg H, Boku N, Bouché O, Catalano P, Fuse N, Michiels S, Moehler M, Morita S, Ohashi Y, Ohtsu A, Roth A, Rougier P, Sakamoto J, Sargent D, Sasako M, Shitara K, Thuss-Patience P, Cutsem EV, Burzykowski T, Buyse M; on behalf of the GASTRIC group.	J Natl Cancer Inst	2013	国外

様式第１９

学　会　等　発　表　実　績

委託業務題目「レジストリーデータの統計解析・活用のためのデータ整備」
機関名：一般社団法人　日本造血細胞移植データセンター

１．学会等における口頭・ポスター発表

発表した成果（発表題目、口頭 ・ポスター発表の別）	発表者氏名	発表した場所（学会等名）	発表した時期	国内・外の別
ホモ接合型HLAハプロタイプを持つ患者の血縁者間造血幹細胞移植におけるHVG方向のみHLA不適合の影響：JSHCT　HLAワーキンググループによる後方視的解析（口頭）	諫田淳也、池亀和博、藤重夫、福田隆浩、黒川峰夫、小川啓恭、大橋一輝、金森平和、石川　淳、井上雅美、一戸辰夫、熱田由子、神田善伸	第37回日本造血細胞移植学会総会	2015	国内
UBMT or immediate UCBT for patients with high-risk AML in first complete remission.(口頭)	Yanada M, Kanda J, Othtake S, Fukuda T, Miyawaki S, Miyamura K, Morishima Y, Kobayashi Y, Atsuta Y, Miyazaki Y, Kimura F, Ohnishi K, Takami A, Naoe T, Kanda Y. UBMT or immediate UCBT for patients with high-risk AML in first complete remission.	第76回日本血液学会学術集会	2014	国内
An allele mismatch has similar adverse impact in related HSCT compared with an antigen mismatch.(口頭)	Fuji S, Kanda J, Miyamura K, Kudo K, Hidaka M, Adachi S, Ichinohe T, Atsuta Y,Kanda Y.	第76回日本血液学会学術集会	2014	国内

２．学会誌・雑誌等における論文掲載

掲載した論文（発表題目）	発表者氏名	発表した場所（学会誌・雑誌等名）	発表した時期	国内・外の別
Impact of HLA Mismatch Direction on the Outcome of Unrelated Bone Marrow Transplantation: A Retrospective Analysis from the Japan Society for Hematopoietic Cell Transplantation. Biol Blood Marrow Transplant.	Kanda J, Ichinohe T, Fuji S, Maeda Y, Ohashi K, Fukuda T, Miyamura K, Iwato K, Eto T, Nakamae H, Kobayashi N, Mori T, Mori SI, Morishima Y, Atsuta Y, Kanda Y; HLA Working Group of the Japan Society for Hematopoietic Cell Transplantation.	Biol Blood Marrow Transplant	2015	国外
Biological significance of HLA locus matching in unrelated donor bone marrow transplantation.	Morishima Y, Kashiwase K, Matsuo K, Azuma F, Morishima S, Onizuka M, Yabe T, Murata M, Doki N, Eto T, Mori T, Miyamura K, Sao H, Ichinohe T, Saji H, Kato S, Atsuta Y, Kawa K, Kodera Y, Sasazuki T.	Blood	2014	国外

Effects of KIR ligand incompatibility on clinical outcomes of umbilical cord blood transplantation without ATG for acute leukemia in complete remission.	Tanaka J, Morishima Y, Takahashi Y, Yabe T, Oba K, Takahashi S, Taniguchi S, Ogawa H, Onishi Y, Miyamura K, Kanamori H, Aotsuka N, Kato K, Kato S, Atsuta Y, Kanda Y.	Blood Cancer J	2014	国外
Comparison of cord blood transplantation with unrelated bone marrow transplantation in patients older than 50 years.	Tanaka M, Miyamura K, Terakura S, Imai K, Uchida N, Ago H, Sakura T, Eto T, Ohashi K, Fukuda T, Taniguchi S, Mori S, Nagamura-Inoue T, Atsuta Y, Okamoto SI.	Biol Blood Marrow Transplant	2014	国外
Decision analysis for donor selection in stem cell transplantation-HLA-8/8 allele-matched unrelated donor vs HLA-1 AG mismatched related donor.	Kanda J, Fuji S, Kato S, Takami A, Tanaka J, Miyamura K, Ohashi K, Fukuda T, Ozawa Y, Kanamori H, Eto T, Kobayashi N, Iwato K, Morishima Y, Sakamaki H, Atsuta Y, Kanda Y.	Blood Cancer J	2014	国外
Impact of HLA allele mismatch on the clinical outcome in serologically matched related hematopoietic SCT.	Fuji S, Kanda J, Kato S, Ikegame K, Morishima S, Miyamoto T, Hidaka M, Kubo K, Miyamura K, Ohashi K, Kobayashi H, Maesako Y, Adachi S, Ichinohe T, Atsuta Y, Kanda Y; HLA Working Group of Japan Society for Hematopoietic Cell Transplantation.	Bone Marrow Transplant	2014	国外
Mycophenolate mofetil use after unrelated hematopoietic stem cell transplantation for prophylaxis and treatment of graft-vs.-host disease in adult patients in Japan.	Iida M, Fukuda T, Uchida N, Murata M, Aotsuka N, Minagawa K, Oohashi K, Fukushima K, Kondo T, Eto T, Miyamoto T, Morishima Y, Nagamura T, Atsuta Y, Suzuki R.	Clin Transplant	2014	国外
Changes in the clinical impact of high-risk HLA allele mismatch combinations on the outcome of unrelated bone marrow transplantation.	Kanda Y, Kanda J, Atsuta Y, Fuji S, Maeda Y, Ichinohe T, Takanashi M, Ohashi K, Fukuda T, Miyamura K, Mori T, Sao H, Kobayashi N, Iwato K, Sawada A, Mori S; for the HLA working group of the Japan Society for Hematopoietic Cell Transplantation.	Biol Blood Marrow Transplant	2014	国外
Continuing increased risk of oral/esophageal cancer after allogeneic hematopoietic stem cell transplantation in adults in association with chronic graft-versus-host disease.	Atsuta Y, Suzuki R, Yamashita T, Fukuda T, Miyamura K, Taniguchi S, Iida H, Uchida T, Ikegame K, Takahashi S, Kato K, Kawa K, Nagamura-Inoue T, Morishima Y, Sakamaki H. and Kodera Y.	Ann Oncol	2014	国外

様式第１９

学　会　等　発　表　実　績

委託業務題目「造血幹細胞移植後にシクロフォスファミドを用いたHLA半合致移植に関する研究」
機関名 筑波大学医学医療系

１．学会等における口頭・ポスター発表

発表した成果（発表題目、口頭 ・ポスター発表の別）	発表者氏名	発表した場所（学会等名）	発表した時期	国内・外の別
骨髄内移植法を用いた臍帯血移植15例の検討	栗田尚樹、横山泰久、関正則、坂田(柳元)麻実子、小原直、長谷川雄一、千葉滋	第37回日本造血幹細胞移植学会総会	2015年3月	国内
同種造血幹細胞移植後の末梢血リンパ球における，Notch分子の発現解析	栗田尚樹、横山泰久、関正則、坂田（柳元)麻実子、小原直、長谷川雄一、千葉滋	第36回日本造血幹細胞移植学会総会	2014年3月	国内

２．学会誌・雑誌等における論文掲載

掲載した論文（発表題目）	発表者氏名	発表した場所（学会誌・雑誌等名）	発表した時期	国内・外の別
Increased serum IgA in Fcα/μR-deficient mice on the (129 x C57BL/6) F1 genetic background	Kurita N, Honda S, Shibuya A	Mol Immunol	2015年2月	国外

様式第１９

学 会 等 発 表 実 績

委託業務題目「a アレムツズマブを用いたHLA不適合移植の開発、 b レジストリーデータの統計解析」
機関名　　自治医科大学

１．学会等における口頭・ポスター発表

発表した成果（発表題目、口頭 ・ポスター発表の別）	発表者氏名	発表した場所（学会等名）	発表した時期	国内・外の別
抗ヒト胸腺細胞免疫グロブリン(ATG: サイモグロブリン)を用いた、進行期造血器腫瘍に対するHLA不適合同種造血幹細胞移植の安全性と有効性に関する後方視的検討、ポスター	賀古真一、赤星佑、原田尚憲、中野裕史、亀田　和明、鵜飼知嵩、山﨑諒子、和田英則、石原優子、河村浩二、坂本佳奈、佐藤美樹、蘆澤正弘、町島智人、寺迫-斎藤桐子、木村俊一、菊地美里、仲宗根秀樹、山崎理絵、諫田淳也、神田善伸	第37回日本造血細胞移植学会総会	2015年3月	国内

２．学会誌・雑誌等における論文掲載

掲載した論文（発表題目）	発表者氏名	発表した場所（学会誌・雑誌等	発表した時期	国内・外の別
Prophylactic role of long-term ultra-low-dose acyclovir for varicella zoster virus disease after allogeneic hematopoietic stem cell transplantation.	Kawamura K, Wada H, Yamasaki R, Ishihara Y, Sakamoto K, Ashizawa M, Sato M, Machishima T, Terasako K, Kimura S, Kikuchi M, Nakasone H, Yamazaki R, Kanda J, Kako S, Tanihara A, Nishida J, Kanda Y.	International Journal of Infectious Diseases	2014.2	国外

Ⅳ.　研究成果の刊行物・別刷

ELSEVIER

Experimental Hematology

Experimental Hematology 2014;42:261–273

Impact of high-/middle-molecular-weight adiponectin on the synthesis and regulation of extracellular matrix in dermal fibroblasts

Hideki Nakasone[a], Kiriko Terasako-Saito[a], Rie Yamazaki[a], Miki Sato[a], Yukie Tanaka[a], Kana Sakamoto[a], Masakazu Kurita[b], Ryoko Yamasaki[a], Hidenori Wada[a], Yuko Ishihara[a], Koji Kawamura[a], Tomohito Machishima[a], Masahiro Ashizawa[a], Shun-ichi Kimura[a], Misato Kikuchi[a], Aki Tanihara[a], Junya Kanda[a], Shinichi Kako[a], Junji Nishida[a], Shigeki Yamada[c], and Yoshinobu Kanda[a]

[a]*Division of Hematology, Saitama Medical Center, Jichi Medical University, Saitama, Japan;* [b]*Department of Plastic Surgery, Kyorin University School of Medicine, Tokyo, Japan;* [c]*Department of Pathology, Saitama Medical Center, Jichi Medical University, Saitama, Japan*

(Received 5 November 2012; revised 16 November 2013; accepted 15 December 2013)

Adiponectin has been shown to play a critical role in immunity. Recently, we reported that the adiponectin levels after allogeneic stem cell transplantation were higher in recipients with chronic graft-versus-host disease (cGVHD). However, the effects of adiponectin on extracellular matrix (ECM) and regulatory factors in dermal fibroblasts remain unclear. We compared the messenger RNA (mRNA) levels of collagen type1 (COL1A), fibronectin 1 (FN1), matrix metalloproteinase (MMP)1, MMP3, tissue inhibitor of metalloproteinase (TIMP)1, TIMP3, transforming growth factor-β (TGF-β), and TGF-β receptor 2 (TGF-βR2) in human normal dermal fibroblasts cultured with and without adiponectin, and we assessed the degree of synthesis of ECMs by immunofluorescent microscopy. Furthermore, we also assessed these mRNA levels after blocking of TGF-βR2. Adiponectin induced higher mRNA levels of FN1, MMP1, MMP3, TIMP1, TIMP3, and TGF-βR2 in a dose-dependent manner, but did not significantly affect COL1A or TGF-β. In addition, adiponectin was shown to upregulate FN1, MMPs, and TIMPs after blocking of TGF-βR2. Immunofluorescent microscopy revealed that adiponectin promoted a greater synthesis of ECMs than in the control in vitro. The finding that adiponectin upregulated ECM-associated factors might mean that high levels of adiponectin could modulate dermal fibrosis was observed in recipients with cGVHD. Further basic investigation is warranted to elucidate whether the adiponectin-pathway could be a target for the treatment of sclerotic cGVHD. © **2014 ISEH - Society for Hematology and Stem Cells. Published by Elsevier Inc.**

Allogeneic stem cell transplantation (SCT) is an important curative treatment for hematologic diseases. However, SCT is associated with many adverse complications, including graft-versus-host disease (GVHD). GVHD is thought to be the result of alloreactive and autoreactive interactions among donor T and B cells, host antigen-presenting cells, and host tissues [1–3]. In particular, chronic GVHD (cGVHD) significantly impairs the recipient's quality of life [4,5]. The detailed mechanism of cGVHD has not been elucidated, although previous reports have investigated various biomarkers for cGVHD [6]. Almost all these biomarkers were shown to be inflammatory cytokines, such as tumor necrosis factor α, soluble interleukin-2 receptor, and soluble B cell activation factor, which are associated with the activation or inhibition of immune cells, including T and B cells [2,7–9]. However, few reports have focused on other endocrine substances in the view of pathophysiology of cGVHD.

Recently, it has been revealed that adiponectin, an adipokine that is secreted by adipose tissues, plays an important role in immunity and inflammation [10–15]. Adiponectin is thought to exist in a globular isoform, trimers, and middle-/high-molecular-weight (MMW/HMW-) multimers. AdipoR1, AdipoR2, and T-cadherin have been identified as specific receptors for each, respectively [16]. The functions of adiponectin are diverse and may depend on the target organ and its isoforms [16,17].

We recently reported that high levels of HMW-adiponectin were observed after SCT in recipients who suffered from cGVHD, and were associated with the severity of cGVHD [18]. However, it is still unclear whether the increase in HMW-adiponectin is a primary or secondary event, as is

Offprint requests to: Yoshinobu Kanda, M.D., Ph.D., Division of Hematology, Saitama Medical Center, Jichi Medical University, 1-847, Amanuma-cho, Omiya-ku, Saitama 330-8503, Japan; E-mail: ycanda-tky@umin.ac.jp

0301-472X/$ - see front matter.
http://dx.doi.org/10.1016/j.exphem.2013.12.009

the role that HMW-adiponectin plays in the pathophysiology of cGVHD. We hypothesized that HMW-adiponectin might have fibrotic or antifibrotic effects on dermal fibroblasts, because skin fibrosis is a major symptom of cGVHD. Skin and organ fibrosis are both defined as the excessive deposition and accumulation of extracellular matrix (ECM), including collagen type 1 and fibronectin [19]. This ECM is produced by dermal fibroblasts, and is known to be increased in scleroderma [20]. The ECMs produced by fibroblasts are regulated by matrix metalloproteinase (MMP), which can degrade ECMs, and by tissue inhibitor of metalloproteinase (TIMP), which can inhibit the activity of MMPs. Therefore, MMPs might improve fibrosis, whereas TIMPs might accelerate the deposition of ECMs and fibrosis [19]. Both MMPs and TIMPs are also produced by fibroblasts.

To date, there has no thorough investigation of the effects of HMW-/MMW-adiponectin on ECM, MMPs, and TIMPs in dermal fibroblasts. Therefore, we assessed the changes in the gene expression of ECMs and regulatory factors, including transforming growth factor (TGF), MMPs and TIMPs, with or without MMW-/HMW-adiponectin in normal dermal fibroblasts in vitro.

Methods

Fibroblast culture

A skin sample was obtained during plastic surgery with informed consent. Superficial dermal samples were incubated with 0.25% trypsin and 0.02% ethylenediaminetetraacetic acid (EDTA) in phosphate-buffered saline (PBS) for 16–24 hours at 4°C, and the epithelium was separated from the superficial dermal sample. Next, human fibroblasts were isolated and cultured for explant at 37°C under a humidified atmosphere of 5% CO_2 in fibroblast growth medium including Dulbecco's modified Eagle's medium with 10% fetal calf serum and 0.6 mg/mL glutamine. Approximately 3 weeks later, primary cultures were subcultured. These dermal fibroblasts derived from a normal subject were used for all experiments during 7–12 passages.

Human recombinant HMW-/MMW-oligomer-rich adiponectin was purchased commercially (BioVendor, Asheville, NC, USA). Fibroblasts were cultured in a humidified atmosphere of 5% CO_2 at 37°C until subconfluence, and harvested with 0.025% trypsin and 0.01% EDTA. At day −1, 5,000 fibroblasts/cm^2 were seeded in each well of an IWAKI 24-well plate in M106 medium (Kurabo, Osaka, Japan) containing 2% fetal bovine serum with 10 μg/mL gentamicin and 0.25 μg/mL amphotericin. After 24 hours (at day 0), each well was washed with PBS and exchanged for 0.5 mL of fresh M106 medium with or without HMW-/MMW-adiponectin.

For the time-dependent assessment, 0 or 10 μg/ml of HMW-/MMW-adiponectin was added to control and target wells, respectively. Fibroblasts were collected at 0, 24, 48, and 72 hours after the addition of adiponectin.

For the dose-dependent assessment, 0, 1, 5, 10, or 20 μg/mL of HMW-/MMW-adiponectin was added to each well. Fibroblasts were collected 3 days after the addition of adiponectin. The adiponectin level in normal subjects is considered to range between 2 and 10 μg/ml [11,16].

Furthermore, we compared the gene expression of ECMs, MMPs, and TIMPs under TGF-β receptor 2 (TGF-βR2)-blocked conditions using 20 μg/ml of anti-human TGF-βR2 antibody (R&D Systems, Minneapolis, MN, USA) and 20 μg/ml of HMW-/MMW-adiponectin. A dose of 10–20 μg/mL of antihuman TGF-βR2 antibody has often been used for the neutralization of TGF-pathways [21]. In the same manner as described earlier, fibroblasts were collected 3 days after cytokine administration. The targets and controls each included two or three wells.

Messenger RNA extraction, complementary DNA synthesis, and quantitative real-time reverse-transcript polymerase chain reaction

After fibroblasts were collected, messenger RNA (mRNA) extraction and complementary DNA (cDNA) synthesis were performed using an RNAspin mini RNA isolation kit (GE Healthcare, Tokyo, Japan) and SuperScript III First-Strand Synthesis SuperMix for qRT-PCR (Life Technologies, Tokyo, Japan) according to the respective manufacturer's instructions. We then performed quantitative real-time reverse-transcript polymerase chain reaction (qRT-PCR) using Taqman Universal Master Mix II (Life Technologies, Tokyo, Japan) according to the manufacturer's instructions. All specific primers and probes for targets and internal control genes were purchased from Life Technologies (Tokyo, Japan): T-cadherin (Hs00169908_m1*), fibronectin 1 (FN1) (Hs01549976_m1*), collagen type I alpha 2 (COL1A2) (Hs00164099_m1*), TIMP-1 (Hs00171558_m1*), TIMP-3 (Hs00165949_m1*), MMP-1 (Hs00899658_m1*), MMP-3 (Hs00968305_m1*), TGF-β1 (Hs99999918_m1), and TGF-βR2 (Hs00234253_m1*). β-actin (433762F) was used as an internal control. All qRT-PCR procedures were performed with a 7900HT FAST Real Time PCR system (Life Technologies, Tokyo, Japan). Relative transcripts were determined by the following formula: $2^{-(\text{CT target} - \text{CT control})}$.

Immunofluorescent microscopy

Observations of ECMs in vitro were performed by immunofluorescent microscopy as described in previous reports [22–24]. At day −1, 5,000 fibroblasts/cm^2 were seeded on cover glasses in IWAKI 35 mm dishes with M106 medium (Kurabo, Osaka, Japan) containing 2% FBS. After 24 hours (day 0), each dish was washed with PBS and exchanged for fresh M106 medium without any cytokines, with 20 ng/mL of TGF-β or 20 μg/mL of HMW-/MMW-adiponectin. At day 4, cells were washed with PBS and fixed with 3.3% formaldehyde of CellFIX (BD Biosciences, Tokyo, Japan) for 15 minutes and 0.5% triton X (Nacalai Tesque, Kyoto, Japan) for 5 minutes. After three washes with PBS, the samples were blocked with PBS containing 2% FBS for 30 minutes. After three washes with PBS, the samples were incubated with primary antibodies of anti-fibronectin IgG produced in rabbit (1:200 in PBS; F3648; Sigma, Tokyo, Japan) and anti-collagen type1 IgG produced in mouse (1:1000 in PBS; C2456; Sigma) at 4°C for 4 hours. After three washes with PBS, anti-rabbit IgG produced in chicken AlexaFluor488 (A21441; Life Technology) and anti-mouse IgG produced in donkey AlexaFluor594 (A21203; Life Technologies) were added (1:500 in PBS for each) and incubated for 30 minutes. ProLong Gold Antifade Reagent with 4′,6- diamidino-2-phenylindoldilactate (Life Technologies, Tokyo, Japan) was added to each sample after three washes with PBS. Images were obtained by laser confocal microscopy (Fluoview Systems FV500; Olympus, Tokyo, Japan).

Immunohistochemistry analysis of skin

Immunohistochemistry (IHC) analyses for fibronectin, MMP-1, MMP-3, TIMP-1, TIMP-3, and TGF-βR2 were performed using formalin-fixed, paraffin-embedded skin samples of a healthy subject and a patient with cGVHD of the skin. In addition, we examined monocyte chemotactic protein-1 (MCP-1) as a representative of proinflammatory cytokines, because it is known to be induced by adiponectin in autoimmune arthritis [11,12]. The samples of a healthy subject were purchased commercially from ILSBio (Chestertown, MD, USA). The skin samples of cGVHD-involved and noninvolved regions were obtained from an autopsy of a patient with skin cGVHD who received steroid administration for 3 years for GVHD and finally died of sepsis-induced thrombotic thrombocytopenic purpura.

Sections (4 μm each) were deparaffinized with xylene and ethanol. Next, the sections were treated with 0.125% trypsin at 37°C for 10 minutes for fibronectin, high pH 9.0 in Tris-EDTA solutions at 97°C for 40 minutes for MMP-3 and TGF-βR2, and low pH 6.0 in citric acid solutions at 97°C for 40 minutes for MMP-1, TIMP-1, TIMP-3, and MCP-1, respectively. Next, they were incubated with primary antibodies at room temperature for 30 minutes in the following dilutions: anti-fibronectin IgG produced in rabbit (1:800, F3648; Sigma), anti–MMP-1 IgG produced in rabbit (1:400, GTX100534; Genetex, Irvine, CA, USA), anti–MMP-3 IgG produced in rabbit (1:1000, GTX100723; Genetex), anti–TIMP-1 IgG produced in mouse (1:200, MS-606-p0; LVC, Fremont, CA, USA), anti–TIMP-3 IgG produced in rabbit (1:400, LLC250885; Abbiotec, San Diego, CA, USA), anti–TGF-βR2 IgG produced in rabbit (1:400, LLC250880; Abbiotec), and anti–MCP-1 IgG produced in rabbit (1:400, 500-P34; Peprotech Rocky Hill, NJ, USA). Thereafter, using EnVision Flex detection system (DAKO, Tokyo, Japan) with Autostainer Link48 (DAKO), the secondary reactions were performed according to the manufacturer's instructions. After peroxidase blocking for 5 minutes, the sections were treated by EnVision FLEX polymer (DAKO). The nuclei were stained with hematoxylin. These views were obtained with Nano Zoomer 2.0RS (Hamamatsu Photonics, Hamamatsu, Japan) and NDP.view2 (Hamamatsu Photonics).

Statistical analysis

Student *t* test and analysis of variance followed by post hoc Tukey multiple comparisons were used for comparisons of mRNA expression in fibroblasts. In addition, the Jonckheere–Terpstra test was used to assess dose dependency. Statistical significance was defined as a two-tailed $p < 0.05$. All statistical analyses were performed with EZR (Saitama Medical Centre, Jichi Medical University; http://www.jichi.ac.jp/saitama-sct/SaitamaHP.files/statmedEN.html) [44], which is a graphical user interface for R (The R Foundation for Statistical Computing, version 2.13.0). More precisely, it is a modified version of R commander (version 1.6-3) that was designed to add statistical functions that are frequently used in biostatistics. This study was approved by the institutional review board of Jichi Medical University.

Results

Expression of T cadherin in fibroblasts

The expression of T-cadherin, the receptor of HMW-/MMW-adiponectin, is known to differ according to the target organ [25]. Therefore, we first confirmed that T-cadherin is expressed in human dermal fibroblasts (Fig. 1A

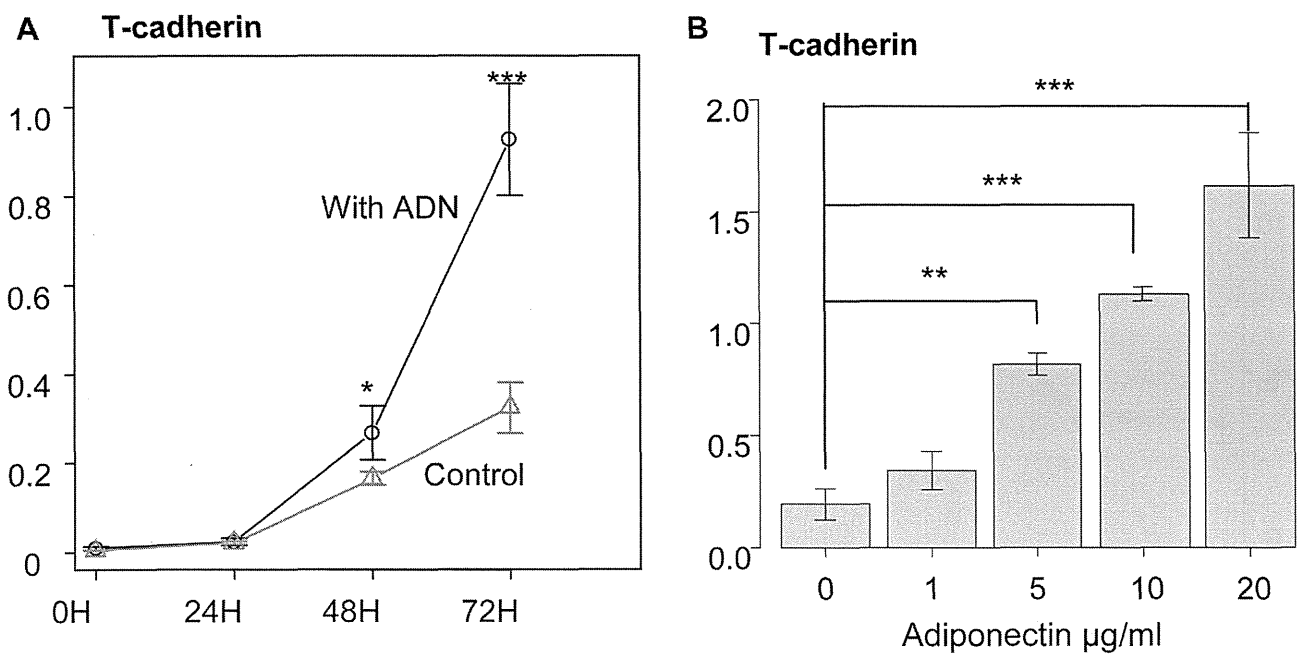

Figure 1. Comparisons of the relative transcripts of T-cadherin. Comparisons of the expression of T-cadherin evaluated by qRT-PCR **(A)** in a time-dependent manner at 0, 24, 48, and 72 hours after 0 or 10 μg/mL of high- or middle-molecular-weight adiponectin administration and **(B)** in a dose-dependent manner 3 days after adiponectin administration (0, 1, 5, 10, or 20 μg/mL). The comparisons are shown between target and control cells. $*p < 0.05$; $**p < 0.01$; $***p < 0.005$.

and B). The expression of T-cadherin at 48 and 72 hours after HMW-/MMW-adiponectin administration was higher than that in controls (Fig. 1A), and this effect was dose dependent ($P < 0.0001$, Jonckheere–Terpstra test; Fig. 1B).

Time- and dose-dependent effects of HMW-/MMW-adiponectin on gene expression in normal dermal fibroblasts

Extracellular matrix expression. Next, we assessed the effect of HMW-/MMW-adiponectin on gene expression for FN1

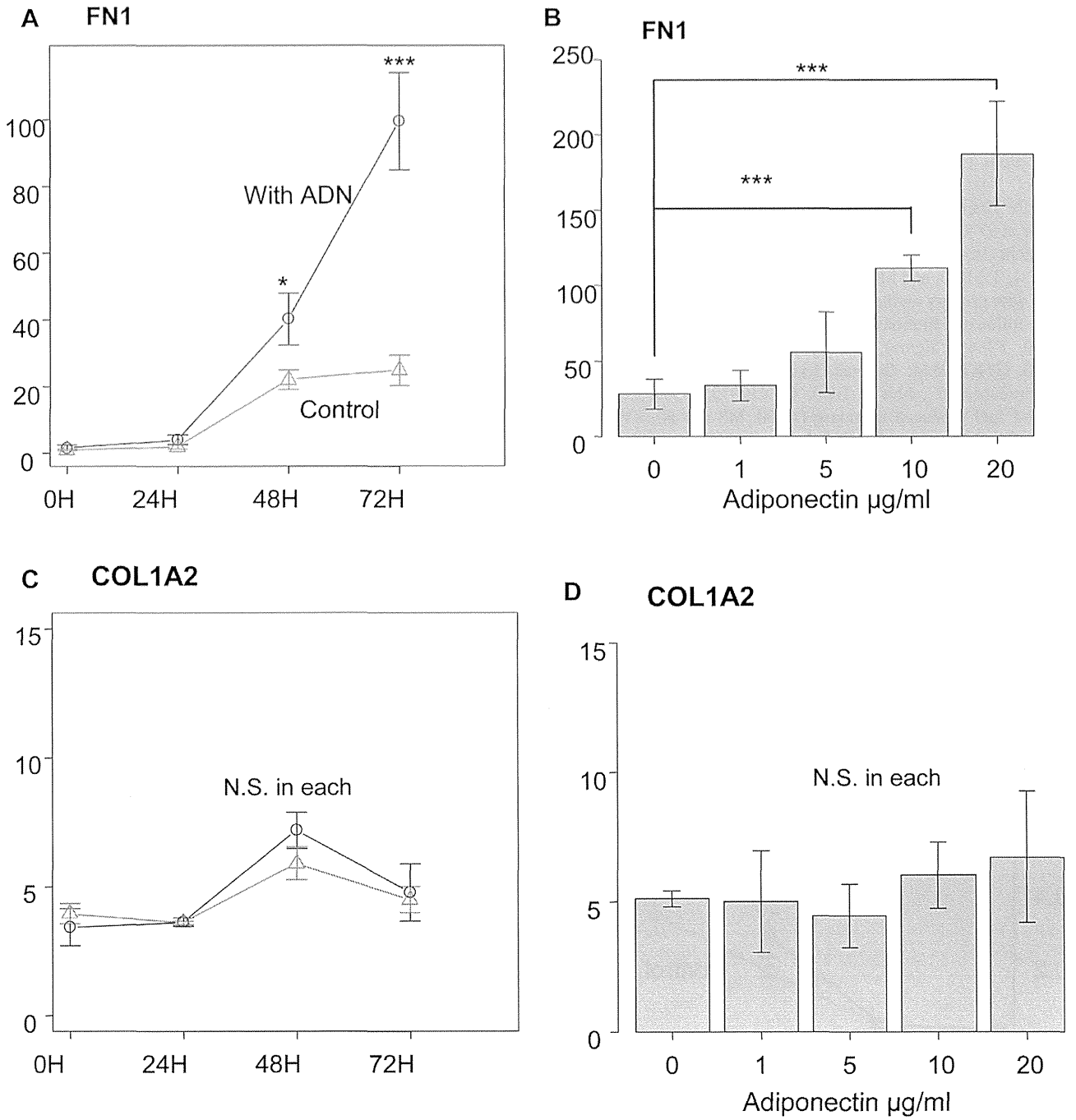

Figure 2. Comparisons of the relative transcripts of extracellular matrix. Comparisons of the expression of extracellular matrix evaluated by qRT-PCR for **(A, B)** fibronectin 1 (FN1) and **(C, D)** collagen type1 alpha2 (COL1A2)—**(A, C)** in a time-dependent manner at 0, 24, 48, and 72 hours after 0 or 10 µg/mL of high- or middle-molecular-weight adiponectin administration and **(B, D)** in a dose-dependent manner 3 days after adiponectin administration (0, 1, 5, 10, or 20 µg/mL). The comparisons are shown between target and control cells. $*p < 0.05$; $**p < 0.01$; $***p < 0.005$.

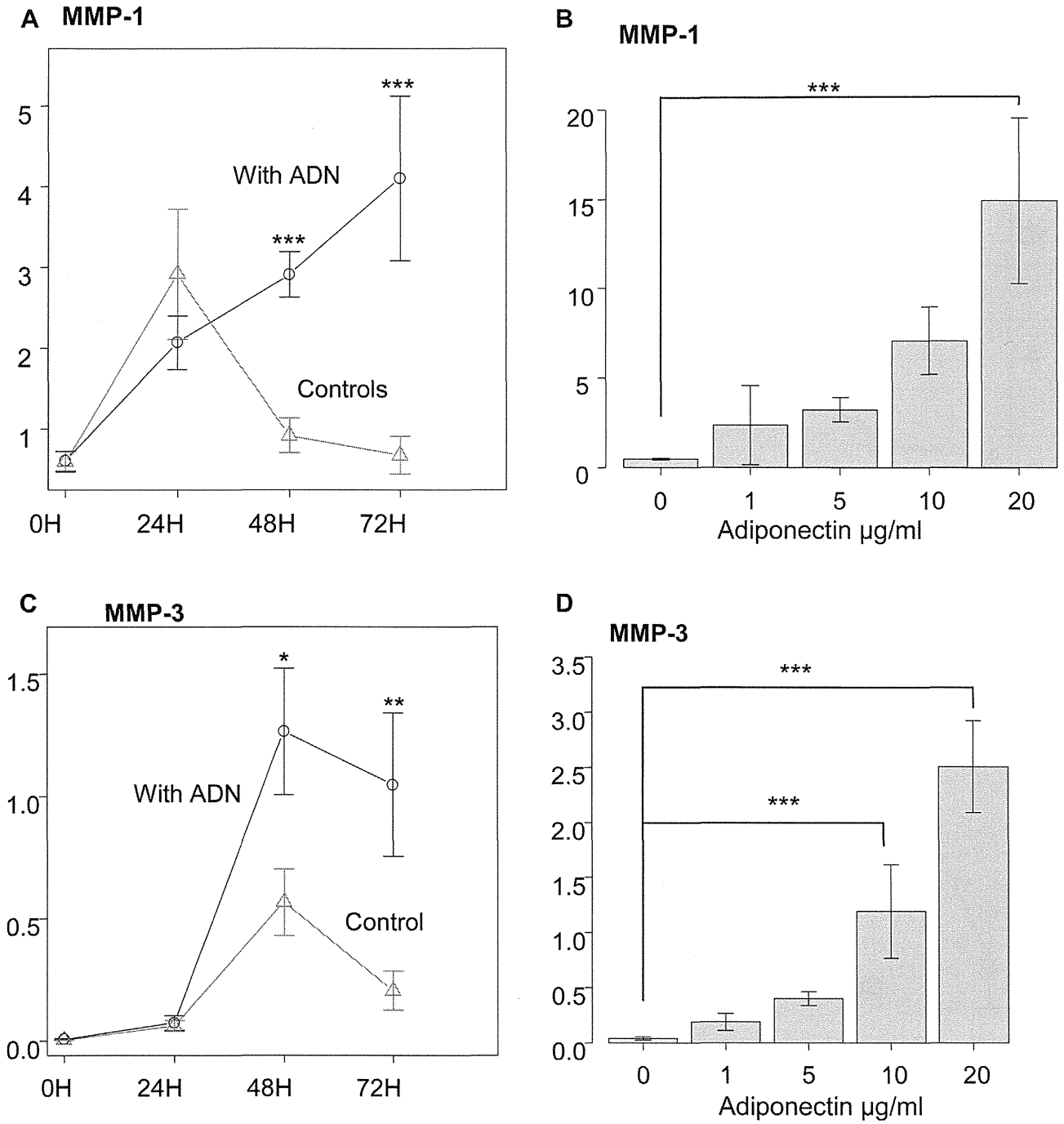

Figure 3. Comparisons of the relative transcripts of metalloproteinase families. Comparisons of the expression of metalloproteinase families evaluated by quantitative real time polymerase chain reaction for **(A, B)** MMP-1, **(C, D)** MMP-3, **(E, F)** TIMP-1, and **(G, H)** TIMP-3—**(A, C, E, G)** in a time-dependent manner at 0, 24, 48, and 72 hours after 0 or 10 µg/mL of high- or middle-molecular-weight adiponectin administration and **(B, D, F, H)** in a dose-dependent manner 3 days after adiponectin administration (0, 1, 5, 10, or 20 µg/mL). The comparisons are shown between target and control cells. $*p < 0.05$; $**p < 0.01$; $***p < 0.005$.

and COL1A2. The expression of FN1 was 2.0-, 1.8-, and 4.0-fold higher than that in controls at 24, 48, and 72 hours after administration, respectively (Fig. 2A). The effect of HMW-/MMW-adiponectin on the expression of FN1 was dose dependent: 1.4-, 3.0-, 4.7-, and 7.8-fold higher than that in the control for fibroblasts cultured with 1, 5, 10, and 20 µg/mL of adiponectin, respectively ($p < 0.001$, Jonckheere–Terpstra test; Fig. 2B). On the other hand, there was no difference in the

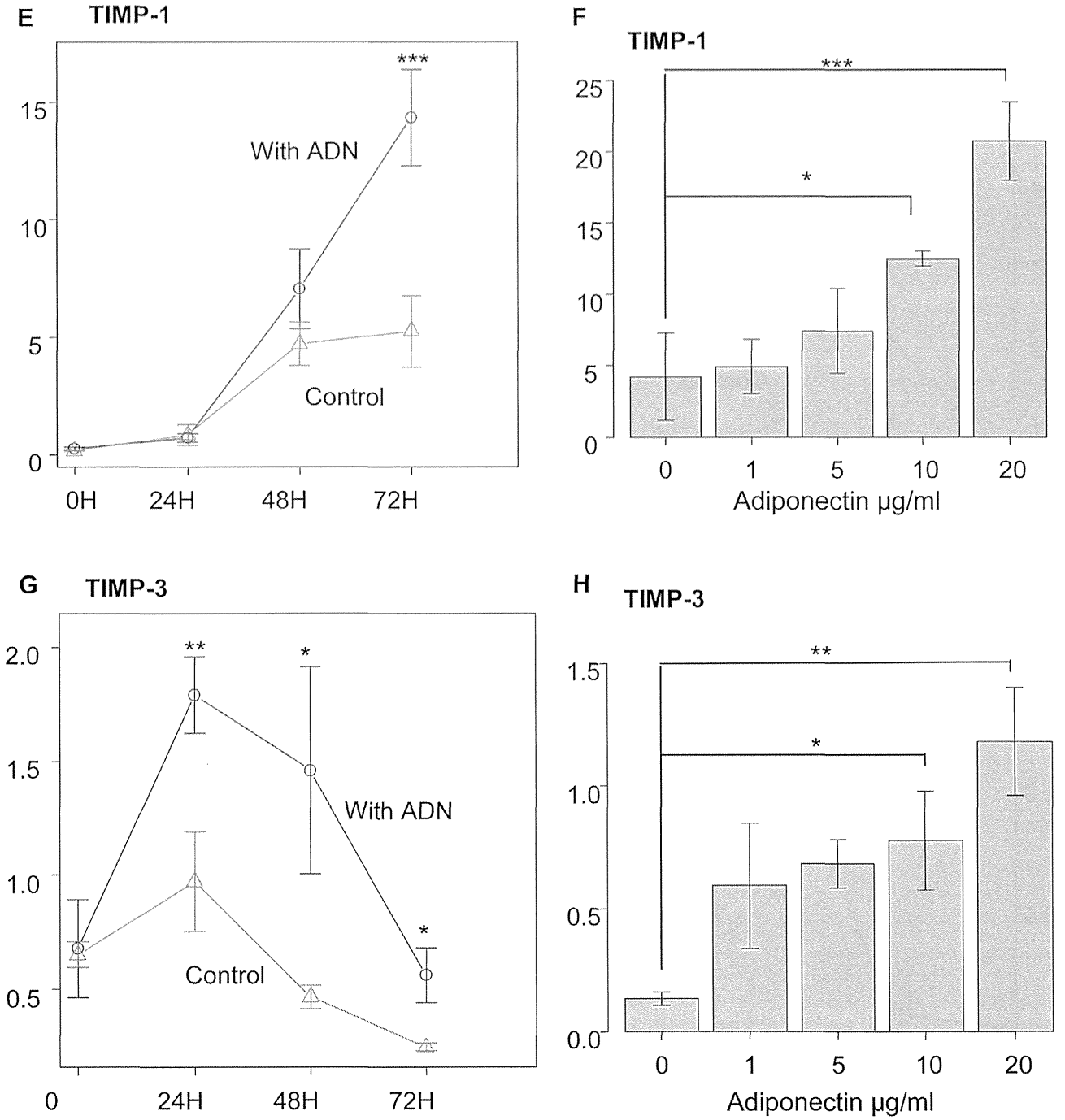

Figure 3. (*continued*).

expression of COL1A2 between fibroblasts with and without HMW-/MMW-adiponectin (Fig. 2C and D).

Metalloproteinase expression. Among metalloproteinase families, we measured the gene expression of MMP-1, MMP-3, TIMP-1, and TIMP-3 because the expression of MMP-1, MMP-3 and TIMP-1 is known to be associated with dermal fibrosis in patients with systemic sclerosis [26]. In addition, TIMP-1 and TIMP-3 are highly expressed in skin involvement of GVHD [27].

The expression of MMP-1 in fibroblasts with HMW-/MMW-adiponectin increased in a time-dependent manner,

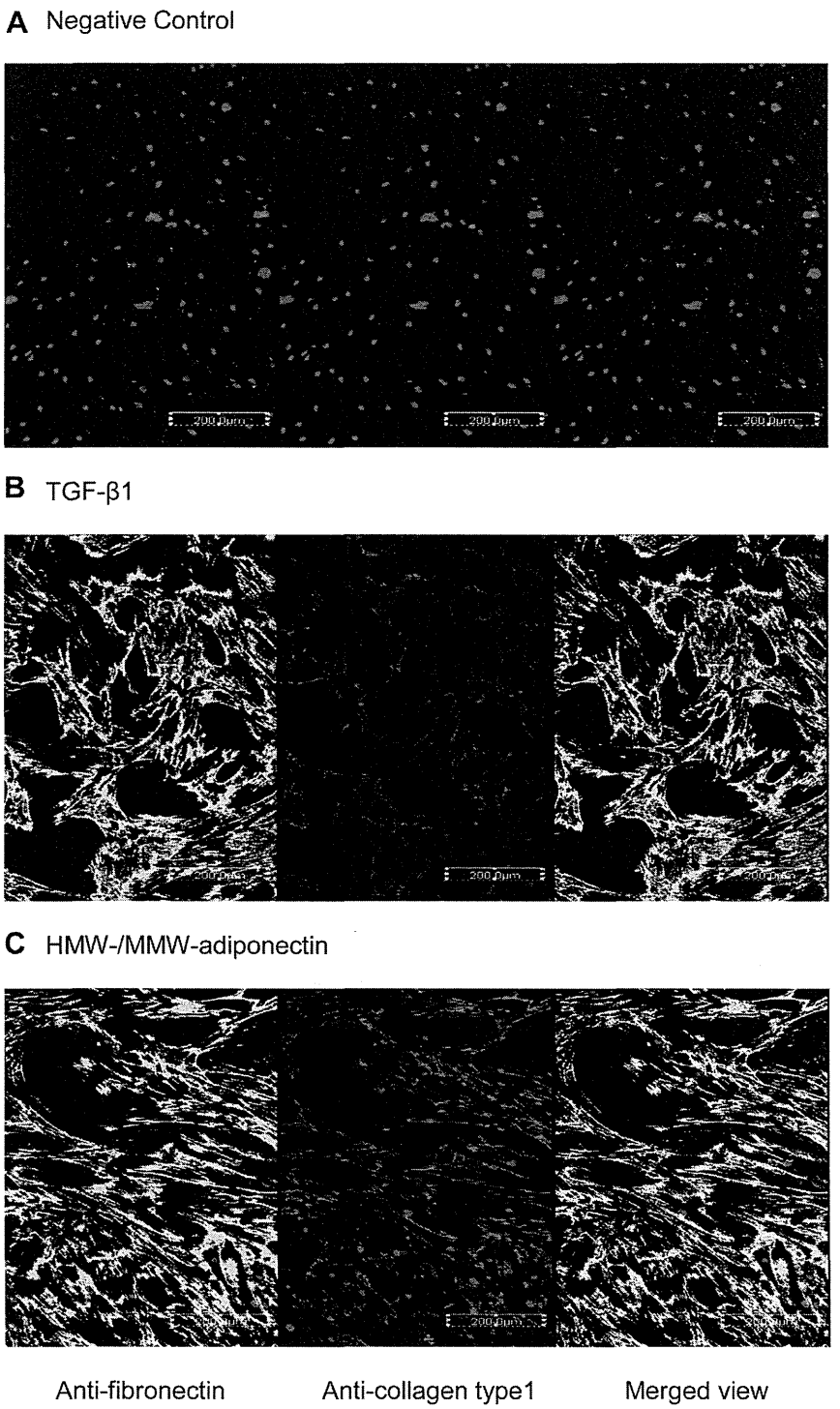

Figure 4. Immunofluorescent microscopy of fibroblasts. Microscopic views 4 days after cytokine administration: **(A)** a control sample without cytokines, **(B)** with 20 ng/mL of TGF-β1, and **(C)** with 20 μg/mL of high- or middle-molecular weight-adiponectin. Green (right), red (middle), and blue (all) signals indicate anti-fibronectin, anti-collagen type 1, and nuclei of cells, respectively. Images on the right are combinations of the other images. Scale bars, 200 μm.

whereas MMP-1 expression in fibroblasts without HMW-/MMW-adiponectin increased 24 hours after administration and then decreased (Fig. 3A). The expression of MMP-1 was dose-dependently higher than that in controls: 5.1-, 7.0-, 15.4-, and 32.6-fold higher in wells with 1, 5, 10, and 20 μg/mL adiponectin, respectively ($p < 0.001$, Jonckheere–Terpstra test; Fig. 3B). The effect of HMW-/MMW-adiponectin on the expression of MMP-3 in fibroblasts

became apparent at 48 hours or later; 1.2-, 2.2-, and 5.1-fold higher than that in controls at 24, 48, and 72 hours after administration, respectively (Fig. 3C), and this effect was dose dependent ($p < 0.001$, Jonckheere–Terpstra test; Fig. 3D).

The effect of HMW-/MMW-adiponectin on the expression of TIMP-1 in fibroblasts was similar to that on the expression of MMP-1 and MMP-3, with an apparent dose-dependent effect at 48 hours or later (Fig. 3E and F). In contrast, the effect of HMW-/MMW-adiponectin on the expression of TIMP-3 appeared earlier: 1.8-, 3.1-, and 2.3-fold higher than that in controls at 24, 48, and 72 hours after administration, respectively, although the total expression started to decrease 48 hours after administration in wells with and without adiponectin (Fig. 3G). The effect of HMW-/MMW-adiponectin on the expression of TIMP-3 was also dose dependent ($p < 0.001$, Jonckheere–Terpstra test; Fig. 3H).

Immunofluorescent microscopy

Based on our observations, we assessed whether HMW-/MMW-adiponectin could produce or degrade ECMs in vitro. Under the current experimental setting, HMW-/MMW-adiponectin seemed to induce greater synthesis and deposition of both fibronectin and collagen type 1 than that in the control, and the effect seemed to be comparable to that of TGF-β1 (Fig. 4).

TGF-β1 and TGF-βR2 expression

Next, we assessed whether HMW-/MMW-adiponectin would produce TGF-β1 and TGF-βR2, a well-known fibrogenic cytokine and receptor. There was no significant difference in the expression of TGF-β1 between fibroblasts with and without HMW-/MMW-adiponectin in this experimental setting (Fig. 5A and B). On the other hand, the expression of TGF-βR2 was 1.9- and 1.6-fold higher than that in the control group at 48 and 72 hours after administration, respectively (Fig. 5C). The expression of TGF-βR2 was dose-dependently higher than that in the control group; 1.6-, 1.8-, 2.2-, and 3.4-fold in wells with 1, 5, 10, and 20 μg/mL of HMW-/MMW-adiponectin, respectively ($p < 0.001$, Jonckheere–Terpstra test; Fig. 5D).

Effect of HMW-/MMW-adiponectin under TGF-βR2–blocked conditions

Next, we assessed whether the effect of HMW-/MMW-adiponectin was dependent on a TGF-β pathway under TGF-βR2–blocked conditions. The increased expression of ECMs, MMPs, and TIMPs by HMW-/MMW-adiponectin was not suppressed by the addition of anti–TGF-βR2 antibody (Fig. 6A–F).

Immunohistochemistry analysis of skin cGVHD

Immunohistochemistry was performed for samples of a normal subject, cGVHD-involved and noninvolved skin region of a patient with skin cGVHD. A diffuse increase of fibronectin expression with strong staining was observed in dermis of the involved region of skin cGVHD compared with normal skin and noninvolved region of skin cGVHD (Fig. 7). TGF-βR2, MMP-3, and MCP-1 were stained mainly in ducts and endothelial cells. An increase of spindle cells that express TGF-βR2 (strong staining), MMP-3 (relatively weak staining), and MCP-1 (weak and small amount) were observed especially in papillary dermis of the involved region of skin cGVHD compared with normal skin and noninvolved region of skin cGVHD (Fig. 7). In addition, they were increased in epidermis of both involved and noninvolved skin regions of the patient compared with normal skin. Cells with MMP-1 expression were also observed in the dermis of the involved region of skin cGVHD, although they were few in number and sporadic with a weak staining (Fig. 7). On the other hand, we could not find any differences in the expressions of TIMP-1 and TIMP-3.

Discussion

Adiponectin has been shown to have both proinflammatory and anti-inflammatory functions [12]. In obesity-related diseases such as diabetes mellitus, adiponectin is thought to induce IL-10, and to have anti-inflammatory effects and protect against cardiovascular events [10,11]. On the other hand, high adiponectin levels have been observed and associated with the disease severity in autoimmune diseases including rheumatoid arthritis, systemic lupus erythematosus, inflammatory bowel disease, and diabetes mellitus type 1 [15,17,28–31]. In addition, adiponectin stimulated the secretion of proinflammatory cytokines, including IL-6, IL-8, and MMPs, in animal models [32]. Although there have been few reports on systemic sclerosis, high adiponectin levels were positively correlated to disease duration and a high skin-thickness score [33]. In addition, it has been shown that the skin of nonobese people, who are thought to have higher adiponectin levels, is thicker than that of obese people [34].

The controversy regarding whether adiponectin has a proinflammatory or anti- inflammatory effect might be due to the fact that most observations and animal models have been based on the use of mixtures of all of the isoforms of adiponectin. Recently, it has been suggested that adiponectin might have different effects on different target cells and tissues, and that its functions might be different according to its isoforms [16,17]. Therefore, we used only HMW-/MMW-adiponectin in our experiments. In addition, no previous study has assessed the effect of adiponectin on not only ECM but also both MMPs and TIMPs in human dermal fibroblasts (Table 1) [35–42]. Therefore, we assessed the effects of HMW-/MMW-adiponectin on human dermal fibroblasts.

The current study showed that HMW-/MMW-adiponectin induced higher gene expression and synthesis of FN1 in dermal fibroblasts. On the other hand, HMW-/MMW-adiponectin did not affect the gene expression of COL1A2, but

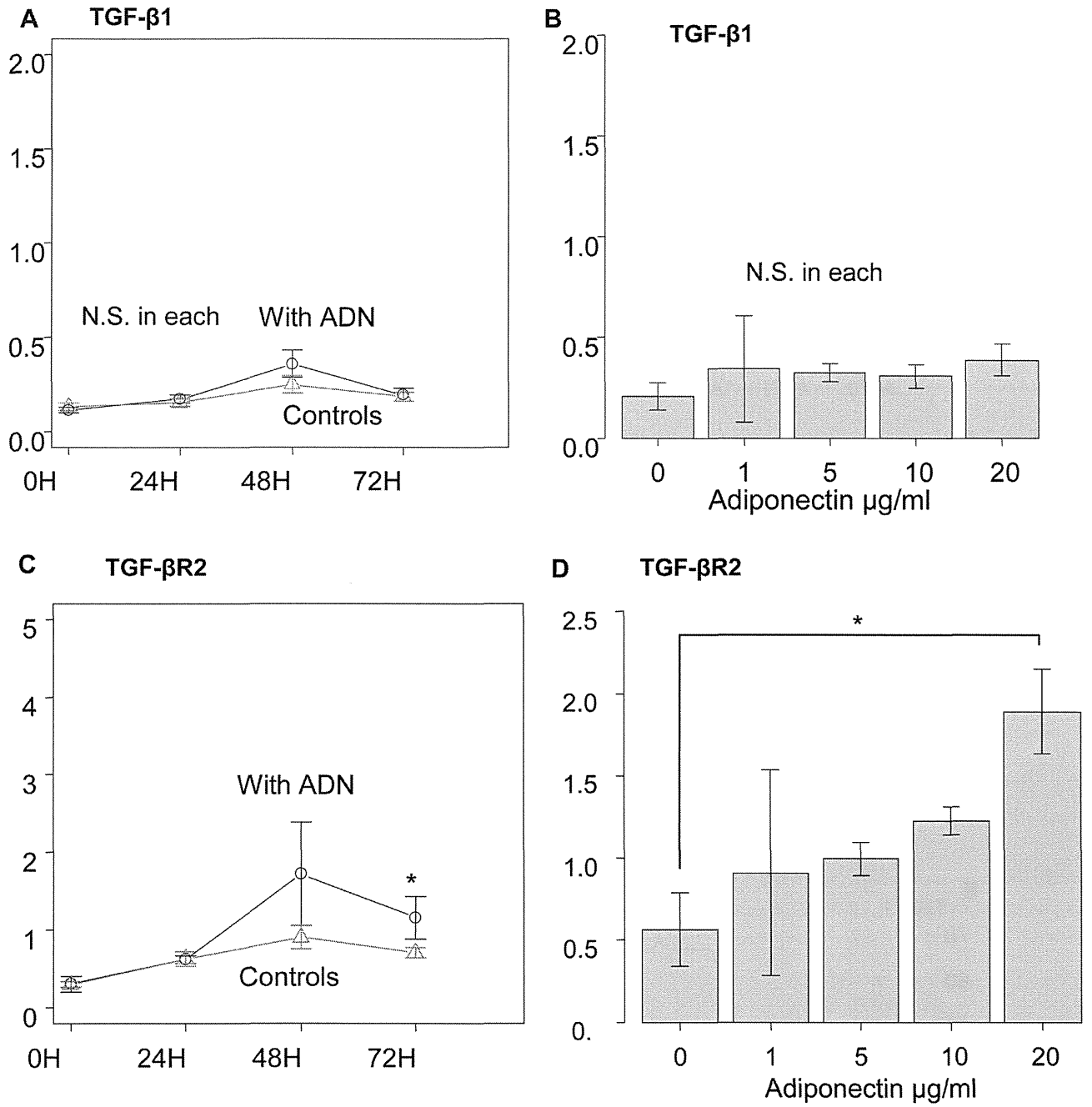

Figure 5. Comparisons of the relative transcripts of TGF-β1 and TGF-βR2. Comparisons of the expression of TGF-β1 **(A, B)** and TGF-βR2 **(C, D)** evaluated with qRT-PCR—**(A, C)** in a time-dependent manner at 0, 24, 48, and 72 hours after 0 or 10 μg/mL of high- or middle-molecular-weight-adiponectin administration and **(B, D)** in a dose-dependent manner 3 days after adiponectin administration (0, 1, 5, 10, or 20 μg/mL). The comparisons are shown between target and control cells. $*p < 0.05$; $**p < 0.01$; $***p < 0.005$.

did induce the greater deposition of collagen type 1 than in the control, which is consistent with a previous report that adiponectin upregulates the secretions but not the gene expression of collagen [43].

It is well known that TGF-β1 is associated with the accumulation of ECMs and fibrosis [19,20]. HMW-/MMW-adiponectin did not have a significant effect on the expression of TGF-β1. On the other hand, it significantly upregulated the expression of TGF-βR2. However, the promoting effects of HMW-/MMW-adiponectin on ECM expression were not suppressed by the neutralization of TGF-βR2. Therefore, HWM-/MMW-adiponectin can increase the expression of ECM independent from the TGF-βR2 pathways.